

Alaska Scientific Crime Detection Laboratory

Quantitative Alcohol Procedure Manual

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SECTION 1: METHODOLOGY FOR ETHANOL QUANTITATION

The Alaska Administrative Code dictates aspects of the collection of blood and method of alcohol analysis. 13 AAC 063.110 gives information on the collection and handling of blood samples and 13 AAC 063.120 details methods of blood alcohol analysis that are appropriate for use in the State of Alaska.

The method used by this laboratory for quantitative ethanol analysis of body fluids and beverages is headspace gas chromatography. A computer-interfaced instrument calibrates and analyzes specimens within a programmed sequence. The result is a comparison of unknown case samples with known concentrations of ethanol, with any variations in amount of injection corrected by the addition of an internal standard, n-propanol.

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SECTION 2: EVIDENCE HANDLING

Evidence submitted to the Laboratory for ethanol analysis includes but is not limited to blood, beverage samples and homebrews. Blood is usually submitted in specific forensic alcohol collection kits containing gray top Vacutainer® tubes. Gray top tubes contain a sodium fluoride preservative and potassium oxalate anticoagulant. Gray top tubes do not require refrigeration because of their preservatives, but collection kits are refrigerated upon receipt to provide extra stability for any unpreserved samples and to comply with the Alaska Administrative Code. Forensic alcohol collection kits are also acceptable for submitting beverage and homebrew samples. Beverage samples are not required to be refrigerated.

Evidence is checked out from the evidence room through the Laboratory Information Management System (LIMS). As with any other type of case the analyst should pay attention to agency numbers, item numbers, laboratory numbers, condition and type of sample as well as the status of tamper-proof seals.

Evidence is stored in a locked refrigerator at all times except during analysis. During analysis the evidence must be secured in the locked blood alcohol laboratory if the analyst is not present.

When opening evidence for analysis each layer of sealed packaging should be marked with the laboratory number, item number, date it was opened and the initials of the analyst who opened it. The tube or item that is to be analyzed should be marked with the laboratory number and item number.

After opening the packaging, each blood sample should be checked for a subject name which should in turn be checked against the Request for Laboratory Services form for accuracy. Any discrepancies or lack of name will be documented in the analyst's notes.

The analyst's notes should also include: how the sample was packaged, if the packaging was sealed, the number and type of tubes submitted, any unusual aspects of the sample, and any broken seals or discrepancies. The date and time of collection and subject name should be recorded if that information is available from the blood tube or sealed container. The analyst should also record the date that the evidence is opened as the start of analysis date in the appropriate field of the notes function in the LIMS. The date of completion field should be left blank until the analyst has completed their analysis and is ready to forward the report on for technical review. The date of completion is entered when the analyst is ready to roll the draft complete milestone in the LIMS.

Outer seals must be resealed after analysis with tamper-proof evidence tape. There should be some means of identifying when and by whom this was performed, usually by initialing and dating the evidence tape. Samples that require toxicology testing should be packaged for shipment to Washington State Patrol Toxicology Laboratory. The outer packaging and any

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additional tubes should be resealed and returned to the evidence section along with all samples that do not require toxicology testing.

Note: Blood and urine are biological materials and precautions associated with handling a biological hazard should be taken. Please refer to the Laboratory's Health and Safety Manual for more information regarding the handling of biological materials.

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SECTION 3: SOLUTIONS, STANDARDS AND CONTROLS

DILUENTS

1. Stock Internal Standard:
Chromatographic grade n-propanol is purchased for use.
2. Working Internal Standard Diluent:
Dissolve 20 grams of NaCl and 0.3 mL of n-propanol in 2 liters of deionized water.
Solution can be stored at room temperature and expires in 3 months.

STANDARDS (CALIBRATORS)

NIST traceable aqueous ethanol standards at 0.025, 0.050, 0.100, 0.200 and 0.300 and 0.400 g/100 mL are used for calibration. Calibration standards are purchased from an ISO 17025 certified supplier. A critical supply vendor form is completed for each standard. The Certificates of Analysis for all standards are kept in the blood alcohol laboratory. Prior to using a new lot of calibrators one vial from the lot should be run as a sample to verify the lot falls within the manufacturer's specifications. The lot numbers and expiration dates should then be entered into the LIMS where they will become part of each case file as a permanent record of the standards used for each calibration.

CONTROLS

1. Negative Control (Blank): Deionized Water
2. Chromatography Control or Volatile Test Mix: To a 500-ml volumetric flask add 500 µl of methanol, acetaldehyde, acetone, ethanol and isopropanol. Dilute to volume with deionized water. The solution can be stored at room temperature and expires in 1 year.
3. Whole Blood Ethanol Controls: Whole blood ethanol controls are purchased from a reputable supplier. A critical supply vendor form is completed for the whole blood controls. The certificates of analysis for all whole blood controls are kept in the blood alcohol laboratory. Prior to using a new lot of whole blood controls one vial should be run ten times to determine the lab parameters of precision. The lab parameters of precision are defined as ± 0.005 or 5% whichever is greater of the mean analyzed value. These values are then checked for agreement with the expected range given by the manufacturer. The values, lot numbers, and expiration dates must be entered into the LIMS where they will become part of each case file as a permanent record of the control used for each batch analysis. If a new shipment of the same lot of controls is received the controls only need to be run once to verify they fall within the prior range.

SECTION 4: QUANTITATIVE ALCOHOL BY HEADSPACE ANALYSIS

Each analysis sequence or run is made up of calibration standards, negative controls or blanks, a mixed volatile chromatography control, whole blood quality control samples and unknown case samples.

Whole blood controls are run every five unknown samples. In addition, a whole blood control should be run before the first unknown sample and after the last unknown sample. Unknown samples and whole blood controls are diluted in duplicate with a diluter/dispenser. The blanks, mixed volatile control and calibrators are diluted singly.

All samples are analyzed by headspace gas chromatography with a flame ionization detector. The instrument uses a computer interface program called Chemstation Headspace® which also generates individual reports for each vial.

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SECTION 5: EQUIPMENT AND INSTRUMENTATION

Diluter/Dispenser

A Hamilton Microlab 500 series diluter/dispenser is used for sample dilution. The NACL dilution method on the diluter/dispenser is set for a 200 µL sample with 2000 µL of diluent along with a 3000 µL wash between samples. The complete parameters for the NACL method can be found in the maintenance and calibration binders for each of the diluter/dispensers.

The diluter/dispenser should be flushed with deionized water after each use. Each diluter/dispenser is sent out annually for calibration and routine maintenance. Documentation of this maintenance and calibration information is kept in binders in the blood alcohol laboratory. Other maintenance and repair is performed, as needed, according to the manufacturer's recommendation and is documented in the maintenance log.

Headspace Gas Chromatograph

An Agilent 6850 gas chromatograph equipped with a G1888 headspace autosampler and a flame ionization detector is used for sample analysis. The instrument is interfaced with a computer and uses the Chemstation Headspace software to calibrate and analyze unknown case samples in the programmed sequence. A printed copy of the current instrument method used for analysis, along with any archived methods, is kept in the binder next to the instrument.

Repair and maintenance of the gas chromatograph and headspace auto sampler is performed as needed in accordance with the manufacturer's recommendations. This is recorded in a binder kept in the instrument room.

SECTION 6: ANALYSIS PROCEDURE

Sample Selection

In the alcohol testing section it is common to receive items that contain multiple containers (units) submitted as a single item. In these instances the analyst must determine which and how many of these units must be sampled and analyzed. The sampling plan listed below describes how the alcohol testing section makes sampling decisions.

Blood samples are usually collected in forensic alcohol testing kits containing two gray top tubes. Blood collected from an individual sequentially into the same type of tube can be treated as one item even when only one tube is sampled. The analyst report will reflect the number of tubes contained in the item along with the result of that item.

Blood collected into different types of tubes will be considered as separate items. The analyst should use their expertise in selecting the best tube for the analysis required and his/her report should indicate only the tube being tested. Any additional tubes in the item can be indicated in the notes field of the LIMS.

Beverage and homebrew samples can often have multiple units collected and submitted as the same item. In many instances the legal needs for the case may only require that one of these units be analyzed. In this case the analyst's report will indicate what was present in each item and what was tested. For example, two 10 mL gray top tubes containing a yellow cloudy liquid were submitted. The analyst would select one tube for analysis and their report would read 2 10 mL gray top tubes of yellow, cloudy liquid one tube analyzed. The ethanol result would then be reported for the tube that was analyzed.

In instances where more than one of the units must be sampled to meet critical volumes listed in AS 04.16.200 the analyst must perform full testing on all units required or use a statistical sampling plan. The statistical sampling plan used by the alcohol testing section is the hypergeometric method. The confidence level associated with this sampling plan must be 95% confidence that at least 90% of the units contain the analyte. The decision about whether full testing is required or a sampling plan is employed will be made on a case by case basis. When a sampling plan is employed details will be provided in the notes of the case record.

Sample Preparation

Remove calibrators, whole blood control(s), and bloods (or beverages) to be analyzed from the refrigerator and allow to warm to room temperature prior to sampling. Glass headspace vials should be set up in the large test tube racks. Prepare two vials for each sample and control that is to be analyzed. Set up one vial each for the blanks, volatile control and calibrators. A blank is run as the first sample in each sequence and a blank and the mixed volatile control is run following the calibration standards. Calibration standards are run at the beginning of each

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sequence and whole blood controls are run after every five case samples and as the first and last unknown sample of each run. A sample blood alcohol sequence can be found in the instrument binder located in the instrument room. Label the autosampler vials, using indelible marker, with the appropriate lab number, calibration level, or control. The autosampler will accommodate 70 vials.

Begin sample preparation by priming the diluter/dispenser with the internal standard diluent. Ensure there is sufficient diluent in the bottle to dilute the entire run. Make sure that there are no bubbles in either syringe. Select the NACI program and follow the instructions as it directs you through the setup. Ensure that each sample is mixed by inverting gently several times prior to sampling. Dispense 2 mL of diluent, along with 200 µL of sample or standard, into the headspace vials using the diluter/dispenser. Wipe the tip of the dispenser between each sample with a Kimwipe. Cover the vials with the caps and crimp tightly onto the vials. Continue this process for all samples in the run.

Beverage and home brew samples that contain ethanol need a preliminary dilution before the diluter/dispenser process. These samples may be diluted 1:100 with deionized water. The dilution factor can then be adjusted by the analyst if needed based on the test results. The resulting ethanol concentration will then need to be multiplied by the dilution used, and then converted to volume of ethanol per volume of liquid by dividing by the density of ethanol. For example:

$$0.050 \text{ g/100 mL (ETOH concentration)} \times 100 \text{ (dilution factor)} \div 0.789 \text{ g/mL (density ETOH)} = 6.3 \text{ mL/100 mL (final ETOH concentration)}$$

In this example, the beverage would be 6.3% ethanol by volume. The “proof” would be twice that, or 12.6.

Instrument Preparation (GC Setup)

The helium for the carrier and makeup gas is supplied from tanks in the vehicle exam room. The hydrogen and air for the flame are supplied by compressed gas tanks located in the instrument room. Periodically check all tanks to ensure the pressure is not below 500 psi. Sequences are imported to the Chemstation software using the LIMS. Instructions on sequence importation and the method parameters are located in the binder next to the instrument. Place all vials into the auto sampler tray in the order they are listed in the sequence table. A review of vial placement must be performed on the run prior to the sequence being initiated. This review will be documented in the operator section of the Sequence Parameters which appears on each chromatogram on the run. Next to the operators name VC and the initials of the person performing the review will indicate the review was performed. The review may be performed by any SCDL employee. If it is not possible to perform a review of vial placement prior to the run being analyzed the analyst may print a copy of the sequence table and have the review performed after the runs completion as long as the vials are not removed from the auto sampler

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tray. This will be documented by the reviewer's initials on the printed sequence table and will be scanned into all the case files in the run.

Software Usage

Sequences are imported into Chemstation using the LIMS. Instructions on sequence importation are located in a binder next to the instrument. For additional instructions on how to use the Chemstation software refer to the manufacturer's instructions.

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SECTION 7: CRIME LAB REPORTS

Upon completion of the run verify that negative control or blanks demonstrate no measurable ethanol and that the volatile mix has baseline separation of all components. Calibrators should read within ± 0.005 or 5%, whichever is greater, of their expected value. The whole blood controls should test within ± 0.005 or 5%, whichever is greater, of the mean analyzed value determined when the lot was run initially. If a whole blood control is out of range, then the samples bracketed by that control must be repeated. If a blank, mixed volatile control or calibrator is out of range the run should be rediluted and rerun.

The two analyzed values for each unknown sample must agree within ± 0.005 or 5% whichever is greater. The reported value is the average of the two analyzed values truncated to three decimal places. For measurement of uncertainty see Appendix A.

None Detected will be reported for analyzed values between 0.000 and 0.009 g/100mL. Linearity is established between 0.025 and 0.400 g/100mL. Alcohol values between 0.009 and 0.025 g/100mL will be reported as less than 0.025 g/100mL. Samples with alcohol values over 0.400 g/100mL will be diluted and rerun.

A LIMS system is used for compiling all case and run data into each case file and generating crime laboratory reports. Instructions for importing data are located in a binder next to the instrument. After the data is imported the analyst should review the electronic case file. When the analyst determines the report is complete and ready for review he/she should record the date in the date of completion field of the notes function in the LIMS. The analyst should then affix their electronic signature.

After reports are signed by the analyst they are forwarded to a qualified person for technical and administrative review.

At the completion of analysis the glass headspace vials should be disposed of in the appropriate biohazard container and the evidence returned to the evidence section if no other testing is required.

SECTION 8: CASE FILES

Case files are stored electronically in the LIMS system. Each case file is composed of:

- A copy of the chromatogram for each duplicate
- A copy of the chromatograms for the blanks, mixed volatile control and each calibrator and whole blood control
- The compound summary of the instrumental run
- The analyst's worksheet
- The Laboratory's Request for Laboratory Services
- Chain of Custody Documentation
- The Crime Laboratory report
- Any case related correspondence

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SECTION 9: PROFICIENCY TESTING

The guideline for satisfactory completion is based on the manufacturer's expected results and the average of all respondents. This means $\pm 10\%$ or ± 2 standard deviations of the average of all respondents for samples. Proficiency test results are maintained by the Quality Manager.

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SECTION 10: CALCULATIONS BASED ON BLOOD ALCOHOL CONCENTRATIONS

It is common for court proceedings to inquire about calculations based on a known blood alcohol result. These include the number of drinks ingested to reach a certain blood alcohol concentration or what a person's blood alcohol level might have been at a prior time. There has been research in the field of alcohol absorption and elimination and there are a variety of methods for performing these calculations. While most methods are reliable and generate similar conclusions one method was selected by this laboratory in an attempt to standardize expert testimony.

Determination of Expected Blood Alcohol Concentration

Absorption of ethanol in the body begins immediately following ingestion. Generally, about 20% of the alcohol consumed is absorbed through the stomach while the remaining 80% is absorbed through the lining of the intestines. Alcohol absorbed through the stomach and intestines enters the blood stream and begins diffusing into the body tissue immediately. The time necessary to reach the peak blood alcohol concentration after drinking ceases varies but on average is near 45-75 minutes.

The formulas used by this lab for calculating an expected blood alcohol concentration are:

Male:

$$(155 \text{ lb/persons weight}) * (0.020) * (\# \text{ of std. drinks}) = \text{Expected BAC}$$

Female:

$$(155 \text{ lb/persons weight}) * (0.024) * (\# \text{ of std. drinks}) = \text{Expected BAC}$$

A standard drink is defined as ½ ounce of pure ethanol, which is equivalent to:

12 oz beer

4-6 oz table wine

1.5 oz of 80 proof distilled spirits

This formula can also be used to determine the approximate number of drinks required to reach a particular blood alcohol concentration.

Retrograde Extrapolation

It is possible for blood alcohol samples to be collected significantly later than the time of the incident; however, in criminal court proceedings the blood alcohol level at the time of incident is often the more significant question. Research in the areas of alcohol absorption and elimination has allowed for qualified personnel to extrapolate backwards from a reliable blood alcohol result

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and make estimations to the blood alcohol level at an earlier time. The limitation of retrograde extrapolation is that the subject must be in the elimination phase of alcohol metabolism (no longer absorbing ethanol) during the timeframe the retrograde extrapolation is being performed.

Elimination of ethanol begins immediately upon entering the blood stream. The body metabolizes about 75-90% of ethanol by converting it to acetaldehyde, then to acetic acid, and ultimately to carbon dioxide and water. Up to 10% of alcohol can be eliminated unchanged through the breath, urine, or other body fluids. Other methods of oxidation account for the remaining elimination of alcohol.

Elimination rates vary between individuals but generally do not vary much in a given individual. The elimination rates used by this lab for retrograde extrapolation are 0.010 – 0.025 g/100mL per hour with an average of 0.017 g/100mL per hour.

Providing Opinions For Court Proceedings

In general, calculations based on a blood alcohol concentration are elicited as expert opinion during testimony in court proceedings. In some instances a written opinion is requested prior to court proceedings. In this case the analyst should prepare a memo containing their opinion and issue it to the requesting agency. A report should not be generated for expert opinion.

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SECTION 11: REFERENCES

1. Alan Wayne Jones, Lars Andersson. Influence of Age, Gender, and Blood-Alcohol Concentration on the Disappearance Rate of Alcohol from Blood in Drinking Drivers. Journal of Forensic Science, Vol. 41 (6): 922-926 (1996).
2. Robert B. Forney Jr. Pharmacology of Alcohol. Handout from The Robert F. Borkenstein Course on Highway Safety. May 2006.
3. Agilent 6850 Gas Chromatograph Manual
4. Agilent G1888 Headspace Autosampler Instruction Manual
5. Hamilton Microlab 500 Series Diluter/Dispenser Manual

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APPENDIX A: UNCERTAINTY OF MEASUREMENT

The forensic alcohol discipline will become compliant with the Supplemental Requirements of the American Society of Crime Laboratory Directors/Laboratory Accreditation Board with respect to measurement of uncertainty before July 1, 2012.

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APPENDIX B: REVISION HISTORY

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